Reproductive and Developmental Effects and Adaptations in Killifish after Chronic, Multigenerational Exposure to Contaminants at a Superfund Site

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ENDOCRINE DISRUPTING CHEMICALS (EDC): AN URGENT ENVIRONMENTAL PROBLEM

The "endocrine disruptor hypothesis" is based on scientific principles; data from laboratory, wildlife and epidemiological studies; weight-of-evidence; and the precautionary principle (See *EDSTAC 1998; Fox et al. 2004).*

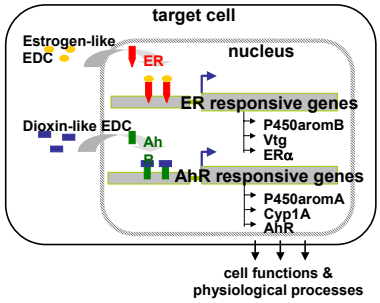


Figure 1. Endocrine discrupting chemicals (EDC). Many different environmental chemicals, by virtue of their ability to bind to hormone receptors, such as estrogen receptors (ER) or arylhydrocarbon receptors (AhR), are able to mimic or block receptor activation of target genes. Other chemicals disrupt normal hormone actions by acting upstream or downstream of receptor binding *per se*. By these mechanisms EDC disrupt cell functions and physiological processes. Very few of the estimated >85,000 chemicals that have been added to the environment have actually been tested for EDC activity, so the extent of the problem is difficult to assess.

SOME UNANSWERED QUESTIONS OF CONCERN

- What are the ultimate consequences of endocrine disruption for individuals, populations, species and ecosystems?
- What are the risks of lifelong exposure to the individual?
- Does exposure during critical developmental stages have permanent effects?
- Do mixtures of EDC have additive, synergistic or antagonistic effects?
- Over multiple generations, do EDC act as agents of selection for genes that promote tolerance, or neutralize or counteract their negative effects?
- If so, are there costs to the individual and the species, including humans?

SCIENTIFIC APPROACH

HYPOTHESIS: Killifish living long-term in highly polluted environments acquire genetic and/or physiological adaptations that neutralize or attenuate endocrine disrupting effects on reproduction & development.

Scorton Creek — New Bedford Harbor

- Reproductive condition
- Molecular markers of estrogen signal transduction
- Functional estrogen response

Figure 2. The killifish population in New Bedford Harbor (NBH) is an extremely valuable research resource. From 1940-1978, NBH became heavily polluted with sewage effluent, PCBs, heavy metals and other industrial contaminants now known to be EDC. NBH was designated a Superfund Site in 1982. Despite >50 yr (>20 generations) pollutant exposure, the NBH killifish population continues to survive and reproduce. Adult and embryonic killifish from NBH were compared with those at a reference site (Scorton Creek, MA) using histological and molecular techniques to assess effects of chronic, multigenerational EDC exposure.

RESULTS

Adult Killifish

Figure 3. Elevated expression of aromatase B (P450aromB), an estrogen responsive gene, in adult NBH fish indicates that NBH is "estrogenic".

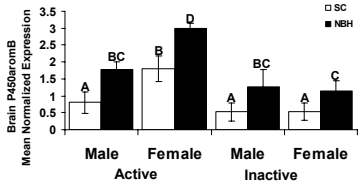
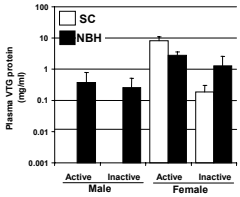


Figure 4. Elevated vitellogenin expression confirms "estrogenicity" of the NBH environment.



Killifish Embryos

Figure 5. ERα, but not P450aromB or vitellogenin, expression is elevated in NBH embryos.

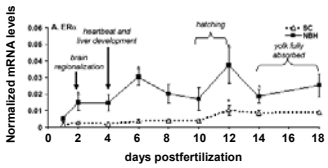


Figure 7. Aromatase B expression is less sensitive to estrogen in NBH embryos.

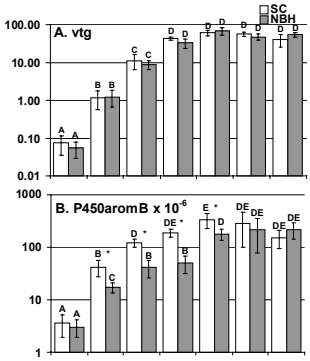
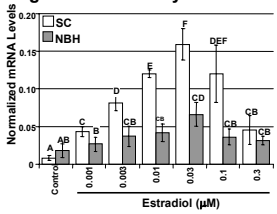


Figure 6. ERα expression is hyporesponsive to estrogen in NBH embryos.



CONCLUSIONS

After long term, multigenerational exposure to high levels of PCBs & other pollutants, NBH killifish embryos and adults:

- Are "estrogenized".
- Show evidence of endocrine disruption, but reproduction & development are not completely blocked.
- Show altered expression of estrogen responsive and reproductively relevant genes.
- Display estrogen hyporesponsiveness of ERα but not other estrogen responsive genes.
- Reveal few (if any) significant site specific single nucleotide polymorphisms (SNPs) in coding regions of Arom or ER genes that could explain aquired estrogen "resistance".

Whether changes in ER expression and "resistance" attenuate effects of NBH pollutants on reproduction & development remains to be determined.

PARTNERSHIPS

- Superfund Basic Research Program (Boston University)
- Woods Hole Oceanographic Institute
- EPA Atlantic Ecology Division (Narragansett RI)

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NIEHS National Institute of Environmental Health Sciences

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